

Trace Back Protocol For Nurseries that Shipped Plant Material to a Confirmed Phytophthora ramorum Infested Nursery 28 September 2006 (Modified February 14, 2008) Version 1.2

Mission/Goal Statement

The goal is to find and eradicate the pathogen if present in nurseries. Any interpretation of this protocol that is contrary to this goal is a misinterpretation of the protocol. A detailed and thorough inspection should take place at the field level to identify the presence of *P. ramorum*. Areas of consideration are to include, but are not limited to, nursery stock, plant material and debris, soil, and water usage.

Purpose

The purpose of this protocol is to establish a set of procedures that are to be used to determine if a nursery that shipped plants to a *Phytophthora ramorum* confirmed positive nursery has infected plants in their inventory, and thus might be a potential source of *P. ramorum*, the plant pathogen that causes sudden oak death, ramorum blight, and ramorum die-back. By following the procedures in this protocol, we can ensure a consistent, science and risk based response to detections of *P. ramorum* in commercial nursery stock.

For more information on this pathogen please visit the USDA, APHIS, PPQ web site at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Definitions

Associated Plants: Associated plants are those reported found naturally infected and from

which *P. ramorum* has been cultured and/or detected using PCR (Polymerase Chain Reaction). For each of these, traditional Koch's postulates have not yet been completed or documented and reviewed

(see Appendix A). (Also see "Host Plants")

Block: Within a nursery, this is a contiguous block of Host and Associated

Host Plants (HAP). The block will be considered contiguous until

there is a 2 meter break of either no plants or no HAP.

Confirmed Positive: The test result on a presumptive positive that *P. ramorum* is present

based on DNA testing or culture morphology. This confirmation would be conducted by APHIS in the case of PASS samples or by the

provisionally approved lab or diagnosticians with identification

authority in the case of non-PASS samples.

Cull Pile: An area where discarded plant material is deposited. Also known as a

waste pile.

Destruction Block: Block of plants to be destroyed. Within a nursery, this is a contiguous

block of HAP containing one or more plants known to be infected with *P. ramorum*. The block will be considered contiguous until there

is a 2 meter break of either no plants or no HAP.

Destruction radius: Block of plants to be destroyed. Within a nursery, for purposes of the

retail protocol, the destruction radius is defined as all *P. ramorum* infected HAP and all other HAP within 2 meters of any infected HAP.

HAP: Host and associated host plants listed on the official "APHIS List of

Regulated Hosts and Plants Associated with *Phytophthora ramorum*".

High Priority Target Plants:

These are any HAP which originated in the destruction block at the infested (source) nursery. These plants are to be identified using the

best available information and to the lowest available taxonomy, (for example, if high risk plants can be identified to cultivar, then trace forward activities may be conducted at the cultivar level). All plants shipped from the infested nursery in the past 12 months which match the description of plants which were in the destruction block are

considered to be high risk.

Host plants: Naturally infected plants verified with completion, documentation,

review and acceptance of traditional Koch's postulates and listed in the "APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*". (Also see: "Associated Plants")

Infected Plants: Plants officially confirmed as being infected with *P. ramorum*, based

on the use of APHIS approved diagnostics, and following the PASS

system.

Medium Priority Target

Plants: Any HAP found in an infested nursery which did not originate in the

destruction block.

Nursery/Facility: Any location where nursery stock is grown, propagated, stored, or

sold; or any location from which nursery stock is distributed. Locations that grow trees to be sold without roots (i.e. Christmas trees) and locations where such trees are stored or distributed are

considered to be nurseries. Also see Retail nursery.

Quarantine Block: Area identified as a 10 meter radius from the destruction block (see

Appendix B) designed to determine if *P. ramorum* has spread beyond

the destruction block. This zone is also known as the quarantine block. (Use of Quarantine Block is an adaptation from the definition: "An area in which a specific pest does not occur, or occurs at a low level and is officially controlled, that either encloses or is adjacent to an infested area, an infested place of production, a pest-free area, a pest-free place of production or a pest-free production site, and in which phytosanitary measures are taken to prevent spread of the pest." [ISPM Pub. No. 10, 1999])

Ouarantine radius:

Block of plants to be guarantined. Within a nursery for purposes of the retail protocol this is an area identified as a 2 meter radius around the destruction radius (see Appendix C) designed to determine if P. ramorum has spread beyond the destruction radius. (Use of Quarantine radius is an adaptation from the definition: "An area in which a specific pest does not occur, or occurs at a low level and is officially controlled, that either encloses or is adjacent to an infested area, an infested place of production, a pest-free area, a pest-free place of production or a pest-free production site, and in which phytosanitary measures are taken to prevent spread of the pest." [ISPM Pub. No. 10, 19991).

PASS (Potentially **Actionable Suspect** Sample):

A presumptive positive P. ramorum sample diagnosed or identified by a provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation. (For more information see: "PASS System Policy" at:

http://www.aphis.usda.gov/plant health/plant pest info/pram/

Retail nursery:

A nursery whose business is the sale of plants to the end user, typically a home owner.

Suspected Infected Plants: These are plants with visible symptoms of *P. ramorum* infection; and/or HAP that are a part of an infested block or derived from an infested block or buffer zone; and/or plants that have tested positive using PCR or culturing, but have not been confirmed positive for P. ramorum by APHIS.

Trace Back (TB) Plants:

All plants of the same taxon (such as genus, species, hybrid, variety, or cultivar) regardless of size, location or lot, back to the original propagation source if still existing.

Trace Back (TB) Site:

Any location that shipped potentially infected plants to a confirmed infested nursery, residence or commercial landscape.

Trace Forward (TF) Plants: Plants identified on a trace forward list as being potentially infected with P. ramorum.

Trace Forward (TF) Site: Any location that received potentially infected plants from a confirmed infested source nursery; including residential or commercial landscapes.

Before Inspection

- 1. An inspection must be conducted when favorable climatic conditions, pathogen infectivity, and host susceptibility occur at the same time. This is commonly in the spring or fall seasons. If conditions are not suitable for disease development at the time of detection, an additional inspection must be conducted when favorable climatic conditions are present.
- 2. For Federal inspectors, notify state officials of your plans to inspect.
- 3. Coordinate visit with State inspector.
- 4. Federal and State or County inspectors should contact the nursery owners/managers prior to the visit to determine how many plants of interest are still in stock and to arrange for the inspection. This nursery contact would normally occur within 24 hours of the expected arrival time for the TB inspection.
- 5. If you are unable to visit the nursery within one day of your contact with the nursery owner/manage, send a PPQ form 523, an Emergency Action Notification (EAN) by fax to them and request that they sign and return it to you by fax.

Survey/Inspection Procedures

- 1. Identify yourself and agency to the nursery/facility owner/manager.
- 2. Explain to the nursery/facility owner/manager the purpose of your visit.
- 3. Determine the presence of any plants of the same taxon (such as genus, species, hybrid, variety, or cultivar) that were reported at the trace forward site.
- 4. Ask owner/manager to fill out questionnaire (see Appendix D), or complete with their input.
- 5. **Sampling method:** Determining the number of plants to be inspected. The goal is targeted sampling of plant tissue to determine the presence of *P. ramorum* with a 95% confidence of finding the disease at a very low level (0.5% of plants are infected with *P. ramorum*) by inspecting a minimum of 850 TB HAP plants in each block (or all the plants if there are less than 850). A physical sample of the inspected plant is only to be taken if unhealthy plant tissue is present. Do not sample plants unless unhealthy tissue is present. If TB HAP plants can not be determined, visually inspect at least 850 HAP plants of the same taxa from each block shipped from the TB nursery. If all TB plants have been sold or are no longer present, have the nursery personnel identify the growing area and then inspect any plants growing in that location.
- 6. Visually inspect the appropriate number of HAP for unhealthy tissue. To visually inspect a plant, carefully lift the plant from surrounding plants and examine all plant leaves and stems for unhealthy tissue particularly for but not limited to the presence of water-soaked or

necrotic lesions consistent with *P. ramorum* infection. Take care to examine the leaves on the interior as they may exist in a microclimate more conducive to disease development and may be more likely to have disease symptoms. Be sure to mark plant as visually inspected either with flagging with the appropriate sample number or a stake with the appropriate sample number. Also examine the leaves that have fallen off the plant for disease symptoms. A physical sample of the inspected plant is only to be taken if unhealthy plant tissue is present. **Do not sample asymptomatic plants**, but feel free to sample any and all plants exhibiting unusual or atypical plant tissue. Images of *P. ramorum* symptoms are available at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/. Keep in mind that these images should only be used as a reference, and should not be considered all inclusive for each species. Links to other sites such as http://www.cnr.berkeley.edu/comtf/, which provide nursery guides that describe and illustrate *P. ramorum* infections are also available at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/. These nursery guides may be viewed and printed from these sites. Symptoms of *P. ramorum* may include:

- Leaf spots
- Twig dieback
- Stem cankers

Keep in mind that many other pathogens cause similar symptoms. Remember that other symptoms caused by *Phytophthora ramorum* as yet unseen may be detected, so sample any unusual or atypical plant symptoms.

7. SAMPLE PLANT TISSUE from any and all visually inspected plants that appear unhealthy. Each sample should consist of a minimum of five leaves; for vaccinium and other small leaf hosts collect the terminal last 3 inches of branch tips, if present, from each unhealthy plant. If, however, only one leaf is symptomatic include only the one leaf with lesions. Please examine any other leaves on the plant for the presence of lesions, because chances are much smaller lesions may be present on other leaves of the same plant. Take care to examine the leaves on the interior as they may exist in a microclimate more conducive to disease development and may be more likely to have disease symptoms, not forgetting the leaves that have fallen off. If certain areas of the nursery are more prone to disease development (such as low areas where water might puddle or places where mist or fog persists) these areas should be included in the sampling process.

For all samples:

- Fill out PPQ Form 391 (Name of host, variety, state code, facility code, etc.).
- Assign a unique sample number using the following conventions: XX-ABC-0001 where XX is your two-letter state code, ABC is a three-letter, state-assigned facility code, and 0001 is the sample number for that facility.
- Log each sample according to the unique sample number.
- Double bag samples (e.g., symptomatic leaf tissue with associated twig intact) in plastic bags.
- Label with collection date, time, location, responsible party. Be sure to write sample number on the bag containing the sample.
- Be sure to mark sampled plants either with flagging with the appropriate sample number or a stake with the appropriate sample number.
- Cool, but do not freeze specimen.
- Submit with minimal delay to your designated laboratory for analysis. All tissue samples must be analyzed by APHIS-approved or APHIS provisionally-approved

- Laboratory using the appropriate diagnostic protocols.
- Overnight the sample if necessary do not send samples on Friday or the day before lab holidays check with the lab as they may not be received until the following Monday. Samples stored without refrigeration may deteriorate and not be testable. Identify the sample(s) as Trace Back (TB) Sample to distinguish the sample(s) from National Survey samples.
- 8. Ask owner/manager to identify cull piles and compost piles. Check any piles for *P. ramorum* symptomatic plants and plant material. Sample as above, if observed.
 - Include appropriate sampling and testing of soil and water. Soil should be tested underneath piles. Water should be baited and sampled from downward slopes, as well as any collected or drained reservoirs from the site.
- 9. If the survey requires the inspector to move among multiple greenhouses, shade houses or discrete blocks, disinfect tools, hands and shoes (or wear disposable gloves and tyvek booties) to prevent pathogen spread between areas. If using disposable gloves and booties, be sure to dispose of these after each individual greenhouse/shade house/block inspection. Disposable rubber gloves and tyvek booties can also be disinfected using 10% bleach solution or a quaternary ammonium solution (at the labeled rate) between inspecting each area. See Exhibit E for details on disinfectants and fumigants for use in nurseries. (Washtubs with ~ 1/2 inch of disinfectant to step in for booties and 3 inches in buckets to dip gloved hands should be sufficient.) Be sure to properly disinfect booties and gloves between all nursery blocks. Disposable gloves and booties should be bagged and disposed by burial or incineration, or in a landfill upon completion of inspections.
- 10. Complete an Emergency Action Notification (EAN, PPQ form 523) or State equivalent, to place a hold on all blocks containing symptomatic plants from the trace back investigation until lab results are released.
- 11. Sanitize/disinfest tools and shoes before leaving premises, using an appropriate disinfectant for the control of *Phytophthora spp.* (such as a 10% solution of bleach or quaternary ammonium solution at labeled rates). See Exhibit E for details on disinfectants and fumigants for use in nurseries.

Laboratory Results/Regulatory Response

- 1. If plant samples are found positive for *P. ramorum* whether the plants are remaining on the site or have been destroyed, either the APHIS, PPQ Confirmed Nursery Protocol for Wholesale and Production Nurseries or the APHIS, PPQ Confirmed Nursery Protocol for retail nurseries will be applied depending if the infested nursery is a wholesale, production or retail establishment.
- 2. If samples associated with the cull or compost piles are found positive for *P. ramorum*, either the APHIS, PPQ Confirmed Nursery Protocol for Wholesale and Production Nurseries or the APHIS, PPQ Confirmed Nursery Protocol for retail nurseries will be applied depending if the infested nursery is a wholesale, production or retail establishment.
- 3. If samples of water or soil are found positive for *P. ramorum*, the Regional Program Manager will provide guidance on proper response.

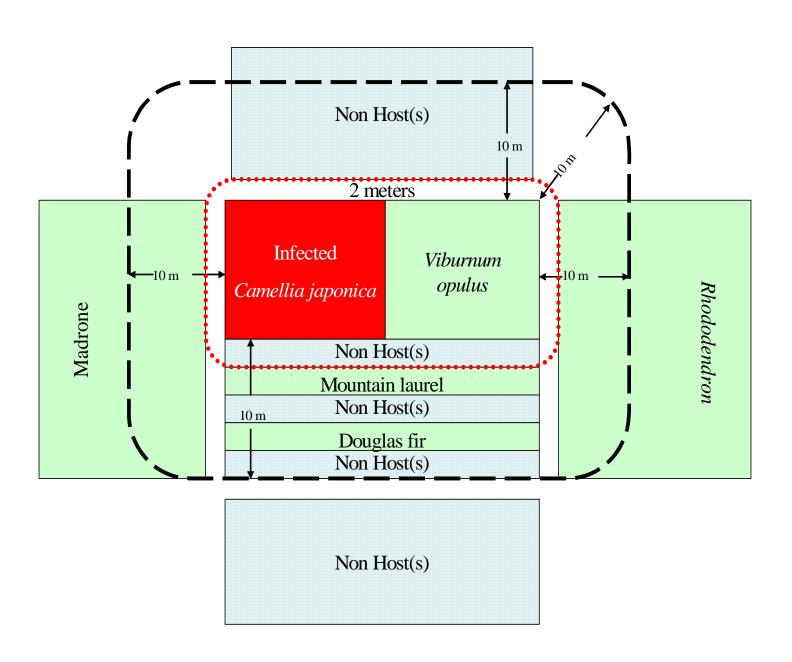
4.	If samples are found negative for <i>P. ramorum</i> , thank the owner/manager of the nursery for their cooperation and release the EAN.

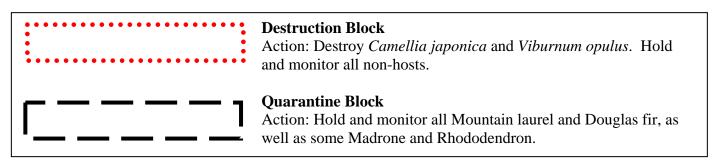
Appendix A

$APHIS \ List \ of \ Regulated \ Hosts \ and \ Plants \ Associated \ with \ \textit{Phytophthora ramorum}$

A current list may be found at the USDA APHIS PPQ website at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Appendix B
Schematic of Nursery with Infected Host Plant(s)

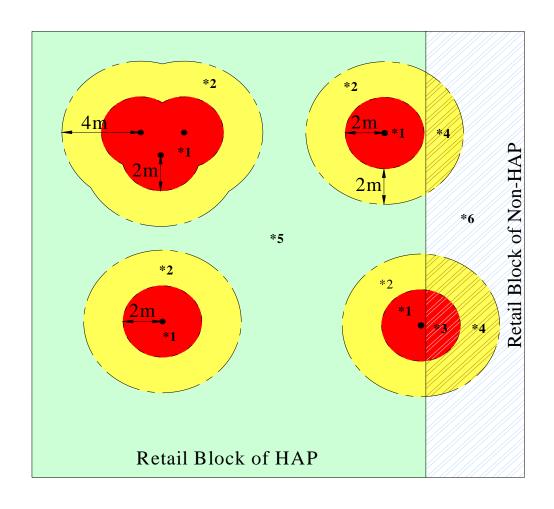




Appendix C

Schematic of Retail Nursery with Infected Host Plant(s)

July 9, 2007



Red (* 1)	Destruction block	Destroy infected plant and all HAP
Yellow (*2)	Quarantine block	Hold HAP from sale for 90 days
Hatch Over Red (*3)		Release non-HAP from sale
Hatch Over Yellow (*4)		Release non-HAP for sale
Green (*5)		Release HAP for sale
Blue Hatch (*6)		Release non-HAP for sale

Appendix D

Phytophthora ramorum Questionnaire (Property Owner or Manager): Part 1

	•		
Name	of Owner or Manage	er:	
Addres	ss of Site:		
City: _		, State: _	, Zip Code:
Contac	ct Name:		, Title:
Phone	Number:		, Fax Number:
GIS C	oordinates (if availal	ole):	
Туре	of Facility (circle):		Greenhouse Wholesale
1.	_	_	he property or facility? If not please provide owner
2.	• •	•	nestion? (If "no", seek information on individual who
3.	How long ago did y	ou purchase the	e plant(s)?
4.	Did you purchase a	ny other plants	from this same nursery?
5.	Have you noticed a	ny other proble	ms with plants on your property?
6.			ved from the infested nursery, from your primary retail
	a) What types and v	•	ars) were they?
	b) How long ago w	as that?	
	c) What is the addre	ess of that locat	ion?

7.	Did you move any plants here from a different location?
	a) What types and varieties were they?
	b) How long ago was that?
	c) What is the address of that location?
8.	Do you have a landscape company that purchases plants from you?
0	What is the contest information for the landscape company?
9.	What is the contact information for the landscape company?
10.	What is your source of water?

Phytophthora ramorum Questionnaire (Property Owner or Manager): Part 2

<u>Information on suspect plant material for inspector visiting property:</u>

1.	What is the variety and number of plants?
2.	What is the condition of the plant material?
3.	Have the plants been trimmed or pruned?
4.	How are the trimmings disposed of?
5.	Did the plant material come in pots?
	a) Did you dispose of the pots or re-use them?
6.	If the pots were reused or stored, describe how the pots were handled?

Appendix E

Treatment and Disinfection

The following techniques are approved by USDA APHIS PPQ for control of *P. ramorum* in nurseries found to contain plants infected with *P. ramorum*.

Infected Plants:

Note: HAP material, including leaf litter, must not be placed in compost piles or be removed from the nursery site as trash or in debris removal. HAP material should be collected and incinerated or double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority.

- Incineration (burning to ash): Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored may be disposed of by incineration at a facility or other location (e.g. on site) approved by USDA and permitted within state and municipal statutes or regulations. Off nursery movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. Burning may be through open burning or in an incinerator.
- **Deep burial:** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored must be double bagged using plastic bags of 2 mil thickness or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or municipal landfill, which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.
- **Steam sterilization:** Dry heat or steam commonly heated to internal temperatures of 212° F (100° C) for 30 minutes followed by burial in a landfill, or as otherwise detailed in the USDA Treatment Manual for "insect pests and pathogens in garbage", Schedule T415b (http://www.aphis.usda.gov/ppq/manuals/pdf_files/Treatment%20Chapters/05-05-T400-5.pdf).

Non-Porous Surfaces:

Most disinfectants are not labeled for use in soil and are only useful for nonporous materials such as concrete floors, nursery pots, and plastic sheeting. A number of disinfectants are registered for use on nonporous surfaces that may effectively reduce populations of *Phytophthora* species. If it is practical, tools such as knives, pruners, water breakers, water wands and other implements used in the quarantine area should only be used in the quarantine area. If tools and other implements must be moved from the quarantine area, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* is recommended prior to removal from the quarantine block. The following table modified from http://cpmcnet.columbia.edu/dept/ehs/decon.html examines the effects of different classes of disinfectants on microbial populations. This list is for explanation and information only. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in **Bold**.

All labels for the disinfectants listed below must be strictly adhered to for maximum efficacy and environmental and worker safety.

Summary of Disinfectant Activities

Disinfectant	Trade names	Comments	Contact time
Alcohols (ethyl and isopropyl) 60-85%	Lysol Spray	Evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable.	10-15 minutes
Phenolics (0.4%-5%)	Pheno-cen	Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue.	10-15 minutes
Quaternary Ammonium (0.5-1.5%)	Consan Triple Action 20 Physan 20 Green-Shield 20	Effective for non-porous surface sanitation (floors, walls, benches, pots). Low odor, irritation. Use according to labels.	10-15 minutes
Chlorine (100-1,000 ppm) 10% Clorox 10% Bleach		Inactivated by organic matter; fresh solutions of hypochlorite (Clorox) should be prepared every 8 hours or more frequently if exposed to sunlight; corrosive; irritating to eyes and skin. Exposure to sunlight further reduces hypochlorite efficacy. Keep solution in opaque container.	10-15 minutes

Water:

- For dust abatement, fire suppression, and equipment cleaning: Clorox (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora lateralis* via water used for dust abatement, fire suppression and equipment cleaning. The active ingredient level must be measured from water collected at the sprinkler head.
- **For irrigation:** Chlorine levels of 2ppm or 2mg/liter or greater has been correlated with the control of *Phytophthora* spp. in re-circulated irrigation systems. For irrigation purposes, recirculated, non-municipal water, must be chlorinated at an active chlorine concentration equal to or greater than 2 mg/liter of water; for facilities that recycle water, this chlorine level must be monitored.

Soil and Potting Media:

- **Potting media:** Potting media must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below.
- Soil: Soil must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below. Methyl bromide has been used for fumigating wood products, but the data on fungi and related organisms in wood are limited. However, methyl bromide has a long history of fumigation of soil in the field and greenhouse. It has commonly been used in combination with chloropicrin for control of *Phytophthora* spp. and other pests in strawberry beds. Methyl bromide has been used for soil treatment for the mitigation of *P. cinnamoni* in citrus groves. However, many of the compounds currently in use have been implicated in human and environmental risks. Solarization is not a consideration as a viable option for soil treatment for *P. ramorum* due to insufficient data on treatment efficacy.

All fumigants are restricted use and must be applied according to labels by a licensed applicator. Any use of pesticides in any manner not listed on the label is unlawful.

Summary of Labeled Soil Fumigants

Fumigant	Trade names	Comments
Chloropicrin	Chlor-O-Pic Metapicrin Timberfume Tri-Clor	Often used in combination with methyl bromide due to its ability to be detected in small quantities.
Dazomet	Basamid	Methyl isothyocyanate (MITC) breaks down into cyanide gas. Granular formulation that is water activated.
Metam-sodium	Busan 1020 Busan 1180 Busan 1236 Metam Vapam	Metam can be applied through irrigation. Tarping can increase efficacy. All application must be made in accordance with labeling.
Methyl Bromide	Tri-Con Terr-O-Gas Preplant Soil Fumigant Pic-Brom	Colorless and odorless. Usually combined in various concentrations with Chloropicrin (tear gas). Use is restricted due to ozone depletion potential.

Physical Treatment of Soil:

• Mitigation of infested soil can also be achieved by installing permanent impermeable, non-porous barriers that consist of cement, concrete or asphalt. These barriers must be constructed so that no native soil within the destruction block is visible. The barriers should be graded such that no standing water can be observed.

Equipment and Personnel (Inspectors and employees):

- Access to infested areas and hold areas should be limited, as much as possible, to officials and employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.
- Personnel should not have access to other parts of the nursery after entering the destruction block on the same day. If entry is unavoidable, follow disinfection procedures in this section.
- A disinfectant foot bath should be placed near the exit to the destruction blocks and quarantine blocks and used by personnel entering and exiting the buffer zone and entering and exiting the quarantine block and entering and exiting the destruction block at the infested nursery site, where the contact with potentially infested soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with filth, in accordance with label directions. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exiting from the quarantine or destruction block. The disposable shoe covers must be placed in bags and incinerated, deepburied or properly disposed in a sanitary landfill.
- The tires (or other parts in contact with the soil or plants, such as the bed of trucks) of vehicles
 must be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products
 before leaving the infested site. If at all possible, vehicles should not be allowed in the destruction
 blocks at all. Any efficacious product labeled for use on non-porous surfaces may be used on tires
 or vehicle undercarriages.
- Do not visit other nursery sites in potentially contaminated work clothing and footwear. Where it is necessary that visitors enter the nursery, the nursery should ensure that every precaution is taken to prevent the movement of infected plants, contaminated soil or debris by the visitor.
- Wood surfaces suspected of contamination with *P. ramorum* should be disposed of as stated above under "Infected Plants". There is no effective way to test or treat wood surfaces for contamination.